Some General Principles of Mutagenicity Screening and a Possible Framework for Testing Procedures

by B. A. Bridges*

It is likely that the assessment of chemicals for mutagenicity will soon become a widespread practice, and a large number of different screening procedures have been proposed. The subjection of every new chemical to be released into the environment to every available test is clearly an impossible task, and it is necessary for an understanding of priorities in terms of risk and benefit to be built into any approach. The present paper represents an attempt to frame a protocol for the assessment of new compounds that is concerned not with the details of individual tests but rather with the questions these tests should be designed to answer and to the evaluation of the answers obtained. It is obviously inherent in such an approach that a similar assessment must be made of chemicals already in the environment, but that is not the purpose of the present article.

Genetic hazards (with the exception of nondisjunction and some other chromosomal abnormalities) are very different from toxic hazards, in that there is little or no likelihood of any feedback from human epidemiological data. Toxicologists have stressed (*1*) that, despite all the animal testing of the past two decades and before, most of what we know about toxic hazards for man has been derived from clinical or epidemiological studies on man himself. Unfortunately, even the overall mutagenic effect of chemicals already in the environment is unlikely to be detectable in man for many generations unless special monitoring procedures are instituted such as electrophoretic analysis of proteins from umbilical blood. Laboratory experiments are therefore even more important in the evaluation of genetic risk than in the evaluation of toxic hazards.

General Principles

In drawing up a framework for the testing and evaluation of chemicals I have been guided by three general principles.

The first is that no generally mutagenic chemical should be released into the environment or be permitted to be used if there exists a satisfactory nonmutagenic substitute. Tests are thus required to detect the mutagenic activity of substances and most of the procedures so far proposed have had this object. The phrase, "general mutagenic chemical," is used specifically to exclude those substances whose mutagenic action is confined to a rather special situation such as formaldehyde when fed to fruit flies. There is likely to be a hazy line between such special mutagens and general mutagens and some knowledge of the mechanism

*MRC Cell Mutation Unit. University of Sussex, Falmer, Brighton BN1 9Q, England.

of mutagenesis will usually be required in order to be able to judge whether man is likely to be affected by a particular agent. The greater value of logical and painstaking in-depth research over routine testing is as obvious here as it is with the assessment of non-genetic toxicological hazards (*2*).

The second general principle is that the extent and rigor of the screening procedures should be related to the extent to which man is likely to be exposed to the agent. Thus a food additive for widespread use would need a great deal more attention than a short-lived no-residue pesticide designed solely for professional horticultural use.

The third general principle is that mutagenic substances may be used if the benefits are judged to be great enough to outweigh the hazards and if appropriate controls are exercised. The precedent for this has already been established by ionizing radiation.

Three-Tier Framework

The proposed framework consists of a three-tiered evaluation procedure (Fig. 1) for both detecting mutagens and evaluating and controlling the hazard from those that are irreplaceable in use.

The First Tier of *in Vitro* Tests

The first tier consists of *in vitro* tests for the production of gene mutations and chromosomal damage. These tests are performed by treatment of various organisms with the chemicals and may be further developed by treatment in combination with various chemical agents and biological materials to mimic the metabolic activation that may occur within the human body.

The precise tests used need not be regarded as immutable and a considerable selection is at present available. As far as the detection of gene mutations is concerned, it would seem that bacterial systems are the most comprehensive and give the most information at the present time (*3–6*). Eukaryotic systems are, however, available and lower eukaryotes (e.g., yeasts, and other fungi), and eventually cultured mammalian cells (*7–10*) may be expected to be developed to the stage where they can yield equally valuable information, although not so cheaply and easily as bacteria. Mitotic recombination in yeasts has also been suggested as an indicator of nonspecific DNA damage (*11,12*). In *Drosophila* the recessive lethal test is elegant and will detect a wide spectrum of point mutations. It is, however, not yet clear how sensitive it is to point mutations at the molecular level, i.e., single base-pair changes, insertions, or deletions.

In vitro systems for detecting chromosome aberrations at metaphase in cultured mammalian cells are now well developed, but it may be almost as convenient to carry out the more recent micronucleus and anaphase bridge techniques on cultured bone marrow cells treated *in vivo* where standard toxicity tests are also to be carried out. Theoretically it should be possible to develop simple tests for detecting agents that give rise to nondisjunction in eukaryotic microorganisms but few have so far been published.

It would seem sensible for *in vitro* tests to be carried out relatively early in the development of a new compound, and since they are simple and cheap it would seem desirable to subject to them not only food additives, pharmaceuticals and pesticides, but also industrial, cosmetic, agricultural and household chemicals. Compounds should be tested at doses well above those to be used in practice as a substance could be used in practice at levels well below those detectable as mutagenic in the laboratory and still, in principle, be a significant hazard to man. Conversely, a valuable compound with mutagenic activity at high doses might still be used if subsequent evaluation showed the hazard at lower doses to be negligible.

It is expected that the majority of chemicals will prove to be negative in these first tier tests, showing that they are not mutagenic *per se*. Those that are not likely to be consumed in any quantity by man (per-

haps most of those entering the body indirectly) would be granted a provisional pass for release. Only those likely to be consumed in significant quantities (including those entering the body directly), such as food additives or medicines, would pass on to the second tier of assessment. Those chemicals giving a positive result would pass directly to the third tier of evaluation.

The Second Tier of *in Vivo* Tests

The second tier of tests would relate to substances designed to be consumed in significant amounts usually directly as food additives and medicines rather than as contaminants. The prime purpose of these tests is to detect substances that are not mutagenic *in vitro*, but are metabolized to an active form *in vivo*. It is true that by not submitting indirectly ingested substances to these tests one may miss a metabolically activated mutagen but since the ingested population dose of such substances would be very small, the population hazard would be minute. It seems far more sensible to concentrate *in vivo* testing on those substances likely to enter the body in significant quantities.

The host-mediated assay (HMA) (*13*) is a test with great potential that will undoubtedly be widely used once the possible interactions of chemicals with the host's response to "foreign" cells are properly understood or bypassed by using cells that are not regarded as foreign. The HMA is only as good as the cellular mutational system that is used, and there is a need for more systems involving the use of mammalian cells. Of course, the HMA should be accompanied by *in vitro* control tests which would give a partial duplication of the first tier examination of this group of compounds.

Production of chromosome aberrations *in vivo* may be tested using either the dominant lethal test in the mouse (*16*) or cytological analysis of short-term cultured bone marrow cells.

As with the first tier, substances that are negative in the tests would be passed for use, and those that are positive in at least one test would pass on to the third tier of evaluation.

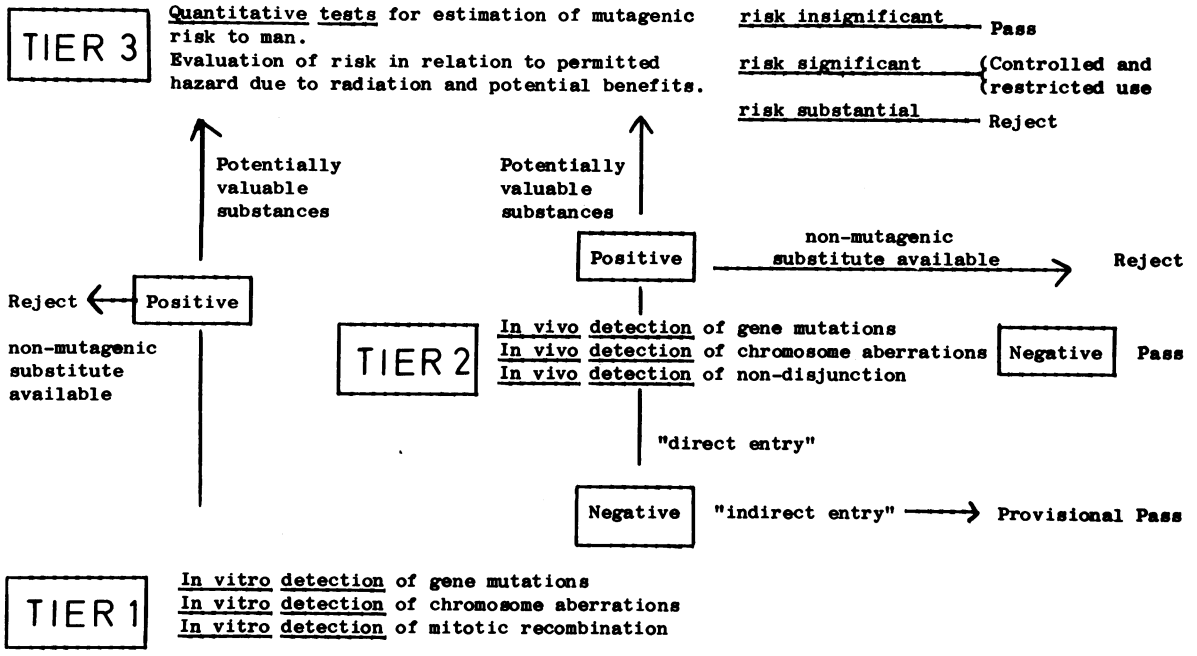

FIGURE 1. Proposed three-tier framework for mutagenicity screening.

The Third Tier of Quantitative Evaluation

Since most compounds giving a positive result at one of the first two tiers would not be essential or unique in their desirable properties they would be forbidden approval on the basis of the first general principle. Some would not be developed further but others with possibly greater potential value might well be considered for further development with the object of producing a nonmutagenic derivative.

We are then left with a group of proven mutagens with potentially great and irreplaceable social, medical or economic value, which must encounter the third tier of assessment. To what extent, if any, should the use of such compounds be permitted in view of the potential accompanying risk? Such a decision has already been made for ionizing radiation so there is nothing new in a risk-benefit assessment in principle. In practice, however, future analyses will differ from that made for ionizing radiation for a number of reasons. Firstly, no chemical can be considered in isolation, nor can chemicals be considered in isolation from radiation, or indeed from naturally occurring mutagens. Secondly, assessment of dose (essential for the evaluation of risk) is likely to be incomparably harder for chemicals than for ionizing radiations.

There might be a small category of substances unlikely to be consumed or absorbed to any extent by the breeding members of the population as a whole, for example certain drugs or industrial chemicals. Compounds in this category might be passed for restricted (and carefully controlled) use among, for example, elderly patients, those with incurable disease, and industrial workers. Such use would, of course, depend upon the evaluation of the carcinogenic risk as being insignificant. Carcinogenicity evaluation should always be carried out rigorously on any mutagen that is proposed for use even among a restricted subpopulation.

The important feature that distinguishes the third from the first two tiers is that whereas the latter comprise tests for the detection of mutagenic activity, the evaluation tier requires that a quantitative estimate be made of the mutational risk to man. This may be an unusual approach to some who have viewed this problem solely from a toxicological angle, but it is a necessary exercise simply because with mutational risks we are concerned with relatively rare events in future populations rather than with observable effects in individual men and women. One cannot assume that a "no-effect" dose level exists for mutagens.

The third tier assessors would be charged with designing the experiments for evaluating the mutational risk. These experiments should take into account the properties and proposed applications of each individual compound. In considering possible experiments, I shall confine my attention to gene mutations only although comparable procedures could be devised for chromosomal damage. In principle, the specific locus test in the mouse (*15*) is a very suitable test that has been successfully used with ionizing radiation. Unfortunately, except in rare cases, the doses of mutagenic compounds to be used in practice would be below the detection level of the test (a level which is in fact determined by the enormous cost of the method). Using higher doses one might be able to obtain values which could be extrapolated to lower doses, but it would be a very laborious business to obtain enough mutants to have any confidence in the extrapolation. I would like to make it very clear that *negative* results in the specific locus test (or the HMA test) do not, because of the low sensitivity of these tests, automatically signify the absence of a hazard for man. A fairly extensive specific locus experiment may well not detect significant mutagenesis from a single dose of 50 rads of gamma radiation, and yet some application resulting in a yearly dose to man 1000-fold smaller than this would be regarded with great concern by the International Commission on Radiological Protection.

The HMA might be used if the cellular system were mammalian and if enough different doses could be used to enable a dose response curve to be obtained. Two disad-

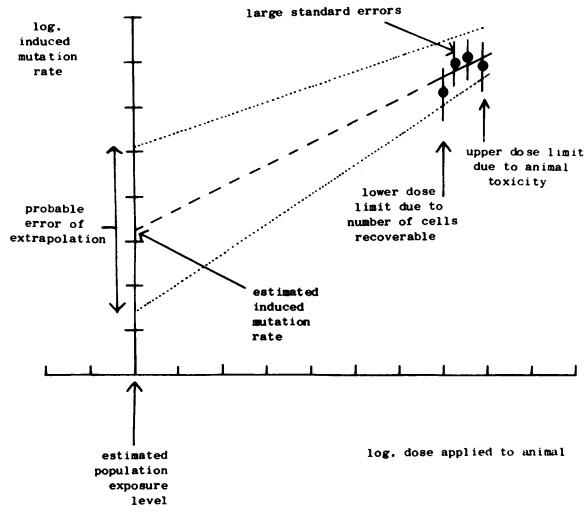

FIGURE 2. Illustration of error resulting from low-dose extrapolation of a hypothetical host-mediated assay experiment.

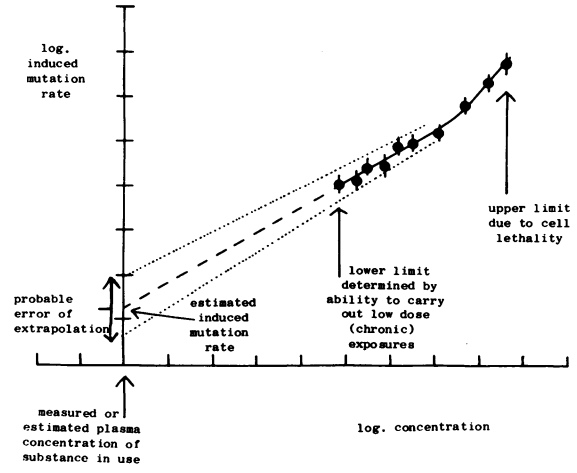

FIGURE 3. Illustration of error resulting from low-dose extrapolation of hypothetical *in vitro* mutagenicity data.

vantages that are immediately apparent are that the variability of response from one animal to another in the HMA would result in very large standard errors, and that the range of usable doses is often small, the lower limit being determined by the number of cells that can be recovered from the animal and the upper limit by toxic effects of the chemical on the animal. Thus the dose response curve would be short and liable to error; extrapolation back would be questionable (Figure 2). It is, however, possible that these problems may be overcome at least with some substances.

These disadvantages would not exist if the dose response curve were determined for mammalian cells *in vitro* (Fig. 3) ideally for a number of different loci and for human as well as rodent cells. Particular attention should be paid to the low-dose region of the dose response curve and, of course, any metabolites active *in vivo* would also need to be studied. It should be possible to extrapolate with a reasonable amount of confidence to population exposure levels expressed as concentrations in plasma, blood, ovary or testis. For some substances, these levels may be directly measurable, for others some extrapolation from higher doses may be necessary. By means such as these it should be possible to obtain a quantitative estimate of mutagenic effect at population exposure doses.

What, then, does one do with such information in order to make an evaluation? I would like to suggest what seems to me to be the most logical course. The most thorough assessment has already been carried out for ionizing radiation and maximum population exposure limits have been laid down. Without in any way regarding these as sacrosanct or immutable one must accept them as being the best at present available. I suggest, therefore, that the effect of a chemical mutagen at proposed population doses should be expressed in terms of the dose of ionizing radiation which would produce the same effect. Such rad-equivalents (radeq units) would probably differ according to the genetic endpoint used; nevertheless an overall value should be calculable. Such a value may then be weighed alongside the present maximum population limits above the background level for ionizing radiation (around 0.17 rad/yr) and the present actual radiation exposures (around 0.01 rad/yr excluding medical exposures

and around 0.035 rad/yr including medical exposures).

The natural background level is around 0.12 rad/yr. Thus the use of a chemical estimated to give a cumulative population exposure to say, >0.1 radeqs/yr might be deemed to be too hazardous to contemplate, whereas one giving <0.005 radeqs/yr might be considered tolerable in view of its advantages and lack of a nonmutagenic substitute. Substances with intermediate values would be recognized as possessing a small hazard that would have to be weighed against the foreseeable benefits. Obviously the outcome of a risk–benefit assessment is likely to vary between one geographical population and another, and between different subpopulations within a given population. It would seem prudent that any mutagenic compound that is assessed as being sufficiently valuable to be released should (a) be given a 5-yr license in the first instance, it being understood that work on a nonmutagenic derivative would be given high priority, (b) have its use limited solely to those situations where its value is deemed to be great, and (c) have its permitted usage levels carefully and quantitatively prescribed.

Obviously the authorized use of any known mutagen should be accompanied by such control procedures as may be required to ensure that all unnecessary exposure is eliminated and that the permitted levels are not being exceeded.

Concluding Remarks

The assessors' task will be frought with difficulties (*16*). One of the more fundamental problems will be the determination of an overall maximum acceptable risk for the human population. Geneticists are far from being agreed on the quantitative aspect of the deleterious consequences of an increased rate of gene mutation and the whole field has developed enormously since the levels for ionizing radiation were set. One thing is patently clear: it is logically absurd and practically foolish to try to deal with genetic hazards from chemicals and from radiations in isolation. Ultimately, we should look forward to the establishment of an Environmental Genetic Hazards Commission to consider environmental genetic damage as a whole, in contrast to the piecemeal assessment of food additives, drugs, pesticides, radiations etc., that exists at present (if it exists at all).

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